

Remarks

I. Status and Nature of the Invention

Claims 1-95 were originally presented. Claims 1-4, 9, 10, 15, 21, 22 and 54-95 have been previously canceled. Accordingly, claims 5-8, 11-14, 16-20, and 23-53 are presently pending. Claims 23-37 are withdrawn from consideration as directed to a non-elected species.

Applicants have amended claim 5 in order to more clearly recite that that a mixture composition comprising a substrate of the enzyme or an analyte compound and an agent that enhances uptake of said substrate or analyte compound is added to the cell sample so that the mixture composition subsides in the medium of the cell sample to form a layer of the mixture composition over the cells, wherein the layer is not homogeneously mixed with the medium. Support for such an amendment can be found, for example, in the last paragraph of Example 1 (paragraph 0149 of the published application US 2003/0077569 A1).

III. The Rejections Pursuant to 35 U.S.C. § 103(a)

A. The Rejections in View of Lucas

Claims 5, 11, 12 and 20 have been rejected pursuant to 35 U.S.C. §103(a) as obvious in view of *Lucas et al.* (U.S. Patent 5,698,411).

The instant invention is drawn to a process wherein a mixture composition, comprising (i) a substrate for an enzyme to be assayed or analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to a cell sample so that the mixture composition forms *a layer* over the cells.

The concept of a layered/non-homogeneous assay mixture was not disclosed or suggested in Lucas. Although Lucas discloses a homogeneous reaction mixture containing

5% DMSO as a solubilizing agent, there is no disclosure or suggestion of the instant invention which is directed to the use of much higher concentrations of DMSO in a mixture with the substrate or analyte compound to provide a mixture of sufficient density so that a layer of the mixture will be formed over the cells. The methods of the invention increase the effective concentration of the substrate or analyte compound in proximity to the cells.

The Examiner's rejection of the claims over Lucas is predicated on the concern that, even-though a layered/non-homogeneous assay mixture is not taught by Lucas, the claims are sufficiently broad that they encompass Lucas because the mixing of intact cells with an assay solution as disclosed by Lucas results in cells that will be covered by, at least in part, the assay solution, thus meeting the "layer" limitation in the claims. Thus the examiner appears to interpret the "limitation" to merely require contact between the cells and the assay components.

To further clarify, Applicant's have amended claim 1 herein to specify that the *mixture composition subsides within the medium of the sample to form a layer of the mixture composition over the cells, wherein the layer is not homogeneously mixed with the medium of the sample.* The claims now clearly distinguish applicant's invention from Lucas wherein assay components were homogeneously mixed with the sample medium to achieve a homogenously mixed assay sample, rather than a stratified assay sample as in the instant invention.

Accordingly, Applicants respectfully submit that the rejection of claims 5, 11, 12 and 20 pursuant to 35 U.S.C. §103(a) as obvious over Lucas may now be properly withdrawn.

B. The Rejections in View of Landrum in combination with Lucas

Claims 5-8, 11-14, 20 and 38-53 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over *Landrum et al.* (U.S. Patent 5,976,822) in view of *Lucas et al.* (U.S. Patent 5,698,411).

The instant invention is drawn to a process wherein a mixture composition, comprising (i) a substrate for an enzyme to be assayed or analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to a cell sample so that the mixture composition forms *a layer* over the cells.

The concept of a layered/non-homogeneous assay mixture was not disclosed or suggested in either the Landrum or the Lucas reference. The assays in Landrum and Lucas were conducted in homogeneous reaction mixtures. Although Lucas discloses a homogeneous reaction mixture containing 5% DMSO as a solubilizing agent, there is no disclosure or suggestion in either Landrum or Lucas of the use of much higher concentrations of DMSO in a mixture with the analyte or substrate to provide a mixture of sufficient density that will form a layer over the cells.

The Examiner's rejection of the claims over Landrum and Lucas is predicated on the concern that, even-though a layered/non-homogeneous assay mixture is not taught by Landrum or Lucas, the claims are sufficiently broad that they encompass Lucas because the mixing of intact cells with an assay solution as disclosed by Landrum or Lucas results in cells that will be covered by, at least in part, the assay solution, thus meeting the "layer" limitation in the claims.

To further clarify, Applicant's have amended claim 1 herein to specify that the mixture composition is added to the sample so that the *mixture composition subsides within the medium of the sample to form a layer of the mixture composition over the cells, wherein the layer is not homogeneously mixed with the medium of the sample*. The claims now clearly distinguish applicant's invention from Landrum/Lucas wherein assay

components were homogeneously mixed with the sample medium to achieve a homogenously mixed assay sample, rather than a stratified assay sample as in the instant invention.

Accordingly, Applicants respectfully submit that the rejection of claims 5-8, 11-14, 20 and 38-53 pursuant to 35 U.S.C. §103(a) as obvious over Landrum in view of Lucas may now be properly withdrawn.

C. The Rejections in View of Zhang

Claims 5-8, 11-14, 20 and 38-53 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over *Zhang et al.* (U.S. Patent 6,248,904).

The instant invention is drawn to a process wherein a mixture composition, comprising (i) a substrate for an enzyme to be assayed or analyte compound and (ii) an agent that enhances the uptake of the substrate or analyte compound into the cells, is added to a cell sample so that the mixture composition forms *a layer* over the cells.

The concept of a layered/non-homogeneous assay mixture was not disclosed or suggested in Zhang. The assays in Zhang were conducted in a homogeneous reaction mixture. Although Zhang discloses that assay mixtures may contain solubilizing agents such as DMSO, there is no disclosure or suggestion in Zhang of sufficiently high concentrations of DMSO in a mixture with the substrate or analyte compound to provide a mixture of sufficient density to form a layer over the cells.

The Examiner's rejection of the claims over Zhang is predicated on the concern that, even-though a layered/non-homogeneous assay mixture is not taught by Zhang, the claims are sufficiently broad that they encompass Lucas because the mixing of intact cells with an assay solution as disclosed by Lucas results in cells that will be covered by, at least in part, the assay solution, thus meeting the "layer" limitation in the claims. The examiner also recites Zhang as indicating particular motivation for the layered arrangement recited in

the claims is that Zhang discloses that the assays may be performed on cells grown in culture in the form of monolayers.

While Applicant's can appreciate the Examiner's position that any solution that is added to cover cells in a monolayer will necessarily form a layer over the cells, Zhang does not in any way suggest the instant invention wherein an assay mixture having a greater density than the cell medium is added to the medium so that the assay mixture subsides within the medium to form a distinct layer over the cells, wherein the layer is not homogeneously mixed with the medium. To further clarify, Applicant's have amended claim 1 herein to specify that the mixture composition is added to the sample so that the *mixture composition subsides within the medium of the sample to form a layer of the mixture composition over the cells, wherein the layer is not homogeneously mixed with the medium of the sample*. The claims now clearly distinguish applicant's invention from Zhang wherein assay components were homogeneously mixed with the sample medium to achieve a homogenously mixed assay sample, rather than a stratified assay sample as in the instant invention.

Accordingly, Applicants respectfully submit that the rejection of claims 5-8, 11-14, 20, and 38-53 pursuant to 35 U.S.C. §103(a) as obvious over Zhang may now be properly withdrawn.

D. The Rejections in View of Landrum, in combination with Lucas and Wansink

Claims 5-8, 11-14, 16-20 and 38-53 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over *Landrum et al.* (U.S. Pat. No. 5,976,822), in view of *Lucas et al.* (5,698,411), and further in view of *Wansink et al.* (1993, *J. Cell Biol.* 122(2):283-293).

The rejection is predicated on the concern that the Landrum/Lucas combination does not disclose the use of glycerol as a solubilizing or permeabilizing agent. Thus, Wansink is cited in conjunction with Landrum/Lucas to provide the suggestion to use glycerol in the reaction mixtures.

Applicants respectfully traverse the rejection. Wansink discloses an assay procedure wherein the assay results are measured on extracellular *precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells rather than on intact cells as in the instant invention. Thus the methods disclosed in Wansink did not require the maintenance of cellular integrity as is required in the instant invention. Thus, one of skill in the art would not have been motivated to combine the teachings of Wansink with the teachings of Landrum/Lucas to arrive at the invention wherein glycerol is the uptake enhancing agent. Furthermore, as discussed above, the Landrum/Lucas combination does not disclose or suggest a layered or stratified assay sample as in the instant invention and Wansink does not disclose or suggest this element of the instantly claimed invention.

Accordingly, Applicants respectfully submit that the rejection of claims 5-8, 11-14, 16-20 and 38-54 pursuant to 35 U.S.C. §103(a) as obvious over Landrum, in view of Lucas and Wansink may now be properly withdrawn.

E. The Rejections in View of Zhang *et al.* in combination with Wansink

Claims 5-8, 11-14, 16-20 and 38-53 have been rejected pursuant to 35 U.S.C. §103(a) as obvious over *Zhang et al.* (U.S. Patent 6,248,904) in view of *Wansink et al.* (1993, *J. Cell Biol.* 122(2):283-293).

The rejection is predicated on the concern that Zhang does not disclose the use of glycerol as a solubilizing or permeabilizing agent. Thus, Wansink is cited in conjunction with Zhang to provide the suggestion to use glycerol in the reaction mixtures.

Applicants respectfully traverse the rejection. Wansink discloses an assay procedure wherein the assay results are measured on extracellular *precipitates* of cellular DNA obtained through the TCA-mediated lysis of the cells rather than on intact cells as in the instant invention. Thus the methods disclosed in Wansink did not require the maintenance of cellular integrity as is required in the instant invention. Thus, one of skill in the art would not have been motivated to combine the teachings of Wansink with the

teachings of Zhang to arrive at the invention wherein glycerol is the uptake enhancing agent. Furthermore, as discussed above, the Zhang combination does not disclose or suggest a layered or stratified assay sample as in the instant invention and Wansink does not disclose or suggest this element of the instantly claimed invention.

Accordingly, Applicants respectfully submit that the rejection of claims 5-8, 11-14, 16-20, and 38-53, pursuant to 35 U.S.C. §103(a), as obvious over Zhang in view of Wansink may now be properly withdrawn.

III. Concluding Remarks

Having now fully responded to all outstanding rejections, Applicants respectfully submit that the present application is in condition for Allowance, and earnestly solicit early notice of such favorable action. The Examiner is respectfully invited to contact the undersigned with respect to any issues regarding this application.

Respectfully Submitted,

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